

100290" 64E93850

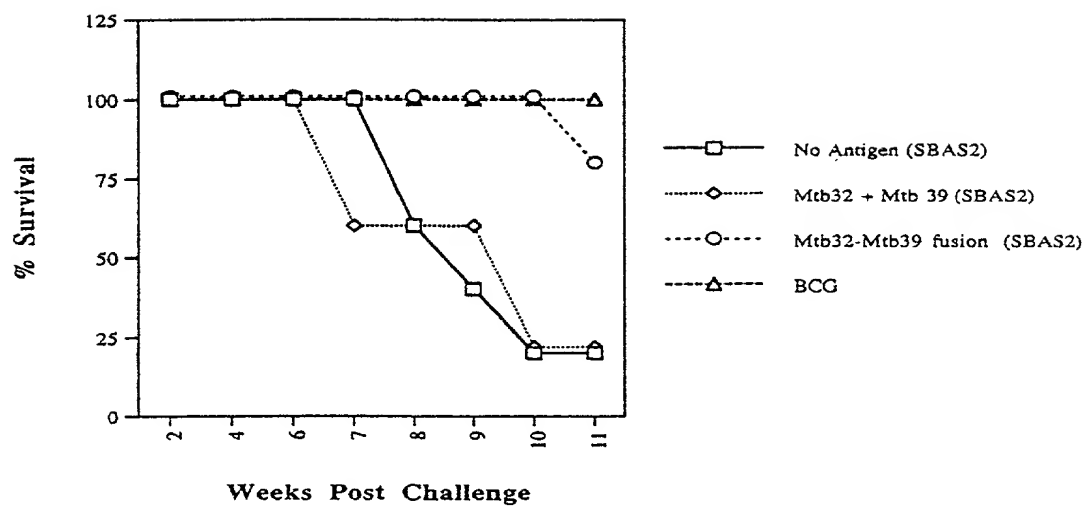


Figure 1

1002591 64E98860

Fig. 2A

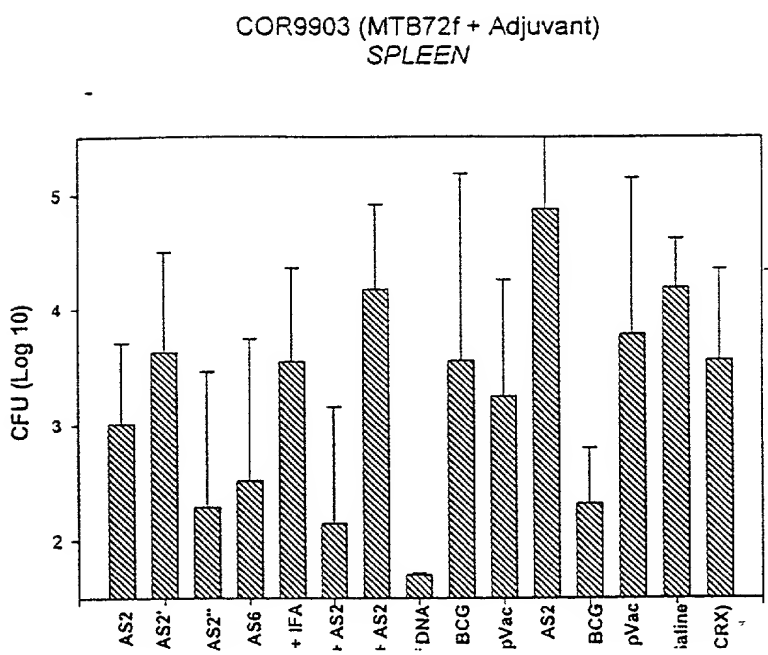


Fig. 2B

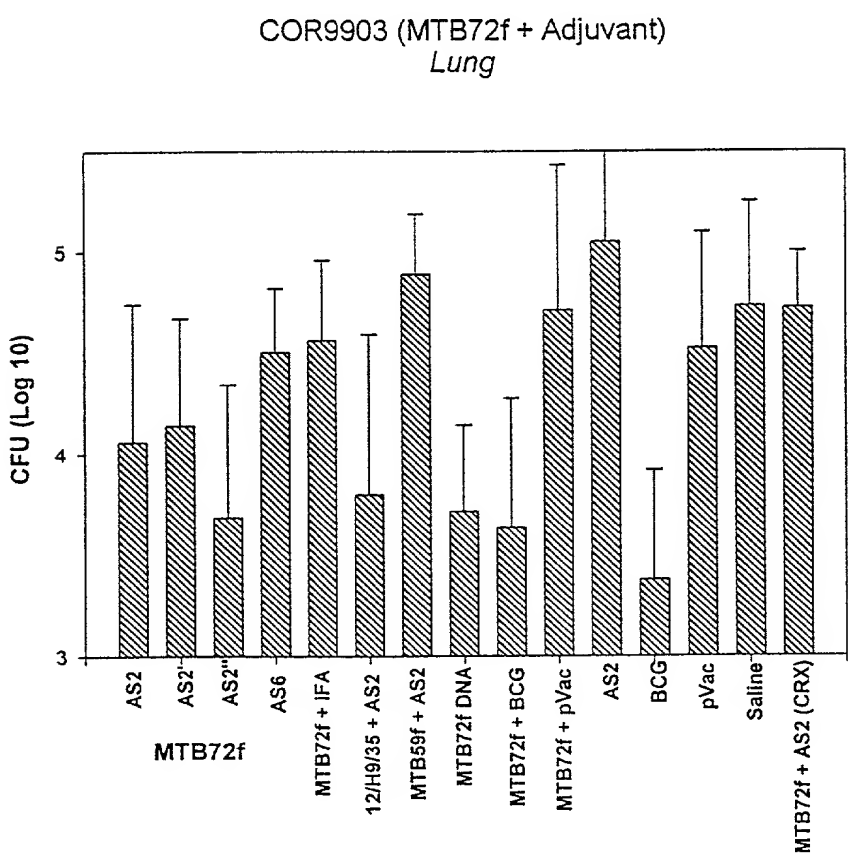
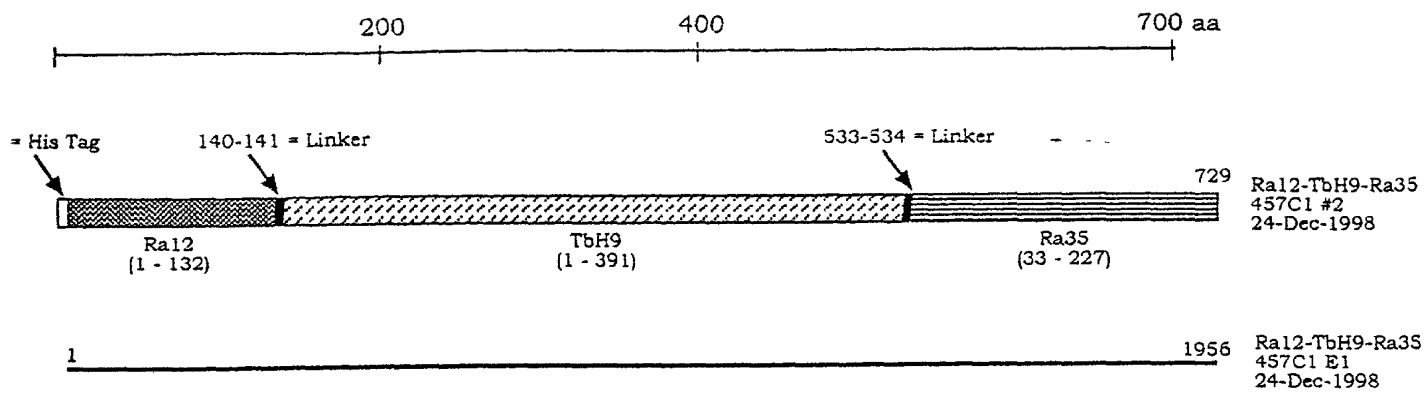


Figure 2

Ra12-TbH9-Ra35
(MTB72f)



0988349-062001-00290-64E98860

Ra35 N-terminus DNA

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gccccgccgg ccttgctgca ggaccgggttc gccgacttcc ccgcgctgcc cctcgaccgc 60
tcgcgatgg tcgcccagt ggggccacag gtggtcaaca tcaacaccaa actgggctac 120
aacaacgcc tgggcgcgg gaccggcatc gtcatcgatc ccaacgggtgt cgtgctgacc 180
aacaaccacg tgatcgcggg cgccaccgac atcaatgcgt tcagcgctcg ctcgggcca 240
acctacggcg tcgatgtggt cgggtatgac cgcaccagg atgtcgcggt gctgcagctg 300
cgcggtgccg gtggcctacc atcggcgggc atcgggtggcg gcgtcgcggt tggtgagccc 360
gtcgtcgca tgggcaacag cgggtgggcag ggcggaacgc cccgtcggtt gcctggcagg 420
gtggtcgcg tcggccaaac cgtgcaggcg tcggattcgc tgaccgggtgc cgaagagaca 480
ttgaacgggt tgatccagtt cgatgccgcg atccagccc gtgattcggg cgggcccgtc 540
gtcaacggcc taggacaggt ggtcggtatg aacacggccc cgtcctag 588

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Ra35 N-terminus amino acid sequence

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Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe Ala Asp Phe Pro Ala Leu
      5                      10                      15

Pro Leu Asp Pro Ser Ala Met Val Ala Gln Val Gly Pro Gln Val Val
      20                      25                      30

Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn Ala Val Gly Ala Gly Thr
      35                      40                      45

Gly Ile Val Ile Asp Pro Asn Gly Val Val Leu Thr Asn Asn His Val
      50                      55                      60

Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe Ser Val Gly Ser Gly Gln
      65                      70                      75                      80

Thr Tyr Gly Val Asp Val Val Gly Tyr Asp Arg Thr Gln Asp Val Ala
      85                      90                      95

Val Leu Gln Leu Arg Gly Ala Gly Gly Leu Pro Ser Ala Ala Ile Gly
      100                     105                     110

Gly Gly Val Ala Val Gly Glu Pro Val Val Ala Met Gly Asn Ser Gly
      115                     120                     125

Gly Gln Gly Gly Thr Pro Arg Ala Val Pro Gly Arg Val Val Ala Leu
      130                     135                     140

Gly Gln Thr Val Gln Ala Ser Asp Ser Leu Thr Gly Ala Glu Glu Thr
      145                     150                     155                     160

Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala Ile Gln Pro Gly Asp Ser
      165                     170                     175

Gly Gly Pro Val Val Asn Gly Leu Gly Gln Val Val Gly Met Asn Thr
      180                     185                     190

Ala Ala Ser
      195

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Figure 4

Figure 5

Alignment of the Amino Acid Sequences of Mtb72f with the the Mutated Version, Mtb72f-mutSA. The single amino acid substitution (S to A) at position 710 is indicated (boxed)

1

1 MHHHHHHHTAASDNFQLSQGGQGFAIPIGQAMAIAGQIRSGGGSPTVHIGPTAFLG Mtb72f
 1 MHHHHHHHTAASDNFQLSQGGQGFAIPIGQAMAIAGQIRSGGGSPTVHIGPTAFLG Mtb72f-mutSA

56 LGVVDNNGNGARVQRVVGSAPAASLGISTGADVITAVDGAPINSATAMADALNGHH Mtb72f
 56 LGVVDNNGNGARVQRVVGSAPAASLGISTGADVITAVDGAPINSATAMADALNGHH Mtb72f-mutSA

111 PGDVISVTWQTKSGGTRTGNVTLAEGPPA]EFMVDFFGALPPEINSARMYAGPGSAS Mtb72f
 111 PGDVISVTWQTKSGGTRTGNVTLAEGPPA]EFMVDFFGALPPEINSARMYAGPGSAS Mtb72f-mutSA

166 LVAAAQMWDSVASDLFSAASAFQSVVWGLTVGWSWIGSSAGLMVAAAAPYVAWMSV Mtb72f
 166 LVAAAQMWDSVASDLFSAASAFQSVVWGLTVGWSWIGSSAGLMVAAAAPYVAWMSV Mtb72f-mutSA

221 TAGQAELTAAQVRVAAAAYETAYGLTVPPPVIAENRAELMILIAATNLLGQNTPAI Mtb72f
 221 TAGQAELTAAQVRVAAAAYETAYGLTVPPPVIAENRAELMILIAATNLLGQNTPAI Mtb72f-mutSA

276 AVNEAEYGEEMWAQDAAAMFGYAAATATATATLLPFEEAPEMTSAGGLLEQAAAVE Mtb72f
 276 AVNEAEYGEEMWAQDAAAMFGYAAATATATATLLPFEEAPEMTSAGGLLEQAAAVE Mtb72f-mutSA

331 EASDTAAANQLMNNVFPQALQQLAQPTQGTTTPSSSKLGGLWKTVSPHRSPISNMVSM Mtb72f
 331 EASDTAAANQLMNNVFPQALQQLAQPTQGTTTPSSSKLGGLWKTVSPHRSPISNMVSM Mtb72f-mutSA

386 ANNHMSMTNSGVSMTNTLSSMLKGFAPAAAAQAVQTAAQNGVRAMSSSLGSSLGSS Mtb72f
 386 ANNHMSMTNSGVSMTNTLSSMLKGFAPAAAAQAVQTAAQNGVRAMSSSLGSSLGSS Mtb72f-mutSA

441 GLGGGVAANLGRAASVGSLSVPQAWAAANQAVTPAARALPLTSLTSA AERGPGQM Mtb72f
 441 GLGGGVAANLGRAASVGSLSVPQAWAAANQAVTPAARALPLTSLTSA AERGPGQM Mtb72f-mutSA

496 LGGLPVGQMGARAGGGLSGVLRVPPRPYVMPHSPAAGD]IAFPALSQDRFADFPAL Mtb72f
 496 LGGLPVGQMGARAGGGLSGVLRVPPRPYVMPHSPAAGD]IAFPALSQDRFADFPAL Mtb72f-mutSA

551 PLDPSAMVAQVGPPQVVNINTKLGYNNAVAGAGTGIVIDPNGVVLTNNHVIAGATDI Mtb72f
 551 PLDPSAMVAQVGPPQVVNINTKLGYNNAVAGAGTGIVIDPNGVVLTNNHVIAGATDI Mtb72f-mutSA

606 NAFSVGSGQTYGVDVVG YDRTQDVAVLQLRGAGGLPSAAI GGGVAVGEPPVAMGN Mtb72f
 606 NAFSVGSGQTYGVDVVG YDRTQDVAVLQLRGAGGLPSAAI GGGVAVGEPPVAMGN Mtb72f-mutSA

661 SGGQGGTTPRAVPGRVVALGQTVQASDSL TGA EETLNGLIQFDAAIQPGD]SGGPVV Mtb72f
 661 SGGQGGTTPRAVPGRVVALGQTVQASDSL TGA EETLNGLIQFDAAIQPGD]SGGPVV Mtb72f-mutSA

716 NGLGQVVGMNTAAS
 716 NGLGQVVGMNTAAS

Figure 6

Alignment of the Amino Acid Sequences of the mature form of Ra35 (Mtb32A) with the mutated version, Ra35 mutSA. The single amino acid substitution (S to A) at position 183 is indicated (boxed)

Ra35 Nterm

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1  MHHHHHHH[APPALSQDRFADFPPALPLDPSAMVAQVGPQVVNINTKLGYNNA TbRa35_mat
1  MHHHHHHH[APPALSQDRFADFPPALPLDPSAMVAQVGPQVVNINTKLGYNNA TbRa35 mutSA

51  VGAGTGIVIDPNGVVLTNNHVIAAGATDINAFSVGSGQTYGVDV-VGY-DRTQ TbRa35_mat
51  VGAGTGIVIDPNGVVLTNNHVIAAGATDINAFSVGSGQTYGVDVVG-YDRTQ TbRa35 mutSA

101 DVAVLQLRGAGGLPSAAIGGGVAVGEPVVAMGNSGGQGGTPRAVPGRVVA TbRa35_mat
101 DVAVLQLRGAGGLPSAAIGGGVAVGEPVVAMGNSGGQGGTPRAVPGRVVA TbRa35 mutSA

151 LGQTVQASDSLGTGAETLNGLIQFDAAIQPGDSGGPVVNLGQVVG-MNTA TbRa35_mat
151 LGQTVQASDSLGTGAETLNGLIQFDAAIQPGD[A]GGPVVNLGQVVG-MNTA TbRa35 mutSA

201 ASDNFQLSQGGQGGAIPIGQAMAIAGQIRSGGGSPTVHIGPTAFLGLGVV TbRa35_mat
201 ASDNFQLSQGGQGGAIPIGQAMAIAGQIRSGGGSPTVHIGPTAFLGLGVV TbRa35 mutSA

251 DNNGNGARVQRVVGSAPAASLGISTGDTVITAVDGAPINSATAMADALNGH TbRa35_mat
251 DNNGNGA-RVQRVVGSAPAASLGISTGDTVITAVDGAPINSATAMADALNGH TbRa35 mutSA

301 HPGDVISVTWQTKSGGTTRTGNVTLAEGPPA]end Ra35
301 HPGDVISVTWQTKSGGTTRTGNVTLAEGPPA]end Ra12
TbRa35_mat
TbRa35 mutSA

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end Ra35 Nterm

Ra12 Cterm

05866349-052001

Figure 7

